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Two new species of *Fulvifomes* (Hymenochaetales, Basidiomycota) from America

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Abstract

Two new species of *Fulvifomes* are described from samples collected in America based on morphological characteristic and molecular evidence: *F. centroamericanus* Y.C. Dai, X.H. Ji & Vlasák, **sp. nov.** and *F. krugiodendri* Y.C. Dai, X.H. Ji & Vlasák, **sp. nov.** The former is characterized by perennial and sessile basidiocarps, a concentrically sulcate and cracked pileal surface, a homogeneous context, small pores (7–9 per mm), a dimitic hyphal system, subglobose, yellowish and thick-walled basidiospores 4.3–5 × 4–4.5 µm. Macroscopically it resembles *F. merrillii*, which differs in having larger basidiospores (5–6 × 4–5 µm). *F. centroamericanus* is similar to *F. robiniae* in sharing applanate basidiocarps and subglobose, yellowish and thick-walled basidiospores 3.9–4.5 × 3.7–4.2 µm, whereas *F. robiniae* has larger basidiospores (5–6 × 4.5–5 µm). In nuclear large subunit rDNA (nLSU) and internal transcribed spacer (ITS) based phylogenies, the two new species formed two distinct lineages in the *Fulvifomes* clade.

Key words

Hymenochaetales, polypore, taxonomy, phylogenetic analysis

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Introduction

Fulvifomes, typified by *F. robiniae*, was proposed by Murrill (1914) to refer to polypores with perennial and sessile basidiocarps, brown and woody context, colored basidiospores, and lacking setae. Several species were referred to this genus by Murrill (1914, 1915), but *Fulvifomes* has been considered as a synonym of *Phellinus* Quél. by many later mycologists (Ryvarden and Johansen 1980, Gilbertson and Ryvarden 1987, Larsen and Cobb-Poule 1990, Ryvarden 1991, Núñez and Ryvarden 2000). Wagner and Fischer (2002) confirmed *Fulvifomes* as an independent genus in Hymenochaetaceae based on nuclear-encoded large subunit rRNA gene (nLSU) sequences. Larsson et al. (2006) acknowledged that *Fulvifomes* was closely related to a clade hosting *Aurificaria luteoumbrina* (Romell) D.A. Reid and *Inonotus porrectus* Murrill with phylogenetic evidence. Dai (2010) included some species with resupinate basidiocarps and/or hymenial setae into *Fulvifomes* without phylogenetic evidence. Zhou (2014) and Hattori et al. (2014) accepted the circumscription of *Fulvifomes* as a genus by the combination of annual to perennial and effused-reflexed, sessile to substipitate basidiocarps with solitary to imbricate pilei and an homogeneous or duplex context, a monomitic to dimitic hyphal system, lack of hyphoid and hymenial setae, and subglobose to ellipsoid, yellowish to brown, slightly thick to thick-walled basidiospores. Recently, several species were introduced to *Fulvifomes* (Hattori et al. 2014, Zhou 2014 and 2015).

During the study on the hymenochaetaceous fungi from North America (USA and Central America), two species belonging to *Fulvifomes* were found with no existing names available for them. Based on both morphology and phylogenetic analyses, they are described as new to science in the present paper.

Materials and methods

Morphological studies

Specimens studied are deposited in the herbaria of the Institute of Microbiology, Beijing Forestry University (BJFC), National Museum Prague of Czech Republic (PRM), and the private herbarium of J. Vlasák (JV). The microscopic procedure follows He and Li (2011, 2013). Special color terms followed Petersen (1996). Spores were measured from sections cut from the tubes. Five % of measurements were excluded from each end of the range, and were given in parentheses. The following abbreviations were used: **KOH** = 5% potassium hydroxide, **IKI** = Melzer's reagent, **IKI–** = neither amyloid nor dextrinoid, **CB** = Cotton Blue, **CB–** = acyanophilous, **L** = mean spore length (arithmetic average of all spores), **W** = mean spore width (arithmetic average of all spores), **Q** = variation in the ratios of L/W between specimens studied, **n** = number of spores measured from given number of specimens.

Molecular study

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain PCR products from dried specimens. Primer pair LR0R and LR7 (Vilgalys and Hester 1990) was used to amplify nLSU sequences, while ITS region was amplified using primers ITS5 and ITS4 (White et al. 1990). The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 54°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR products were purified and sequenced in Beijing Genomics Institute, China, with the same primers.

Phylogenetic analysis

In this study, thirteen new sequences were generated (Table 1). Other sequences for phylogenetic analysis were downloaded from GenBank which were used in the previous study (Zhou 2015), the nLSU dataset with *Stereum hirsutum* (Willd.) Pers. and *Bondarzewia montana* (Quél.) Singer as the outgroup (Wagner and Fischer 2002) was used to confirm the generic position of the newly sequenced specimens. The ITS dataset was used to further clarify the interspecific relationships of *Fulvifomes* with *Phellinus laevigatus* (P. Karst.) Bourdot & Galzin, and *P. populicola* Niemelä as the outgroup (Wagner and Fischer 2002).

Sequences were aligned with BioEdit (Hall 1999) and ClustalX (Thompson et al. 1997). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps were manually adjusted to optimize the alignment. Sequence alignment was deposited at TreeBase (submission ID 19989; www.treebase.org). Phy-

Table 1. Information for the newly sequences used in this study.

Species	Location	Sample no.	GenBank accessions	
			ITS	nLSU
Fulvifomes centroamericanus	Costa Rica	JV1408/4	–	KX960768
F. centroamericanus	**Guatemala**	**JV0611/III**	**KX960763**	**KX960764**
F. centroamericanus	Guatemala	JV0611/8P	KX960757	–
F. grenadensis	USA	JV1212/2J	KX960756	–
F. grenadensis	Costa Rica	1607/66	KX960758	–
F. krugiodendri	**USA**	**JV0904/1**	**KX960762**	**KX960765**
F. krugiodendri	USA	JV0312/24.10J	KX960760	KX960766
F. krugiodendri	USA	JV1008/21	KX960761	KX960767
Inonotus porrectus	Costa Rica	1412/6J	KX960759	–

Type species are shown in bold.

logenetic analysis was carried out according to previous studies (Zhou 2015). Maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) were employed to perform phylogenetic analysis of the two aligned datasets. The three phylogenetic analysis algorithms generated nearly congruent topologies for each dataset, and, thus, only the topology from the MP analysis is presented along with statistical values from the ML, MP and BI algorithms (simultaneous BS not less than 50 % and BPP not less than 0.8) at the nodes.

Results

The nLSU-based phylogeny (Fig. 1) showed that the newly sequenced specimens fell into the strongly supported *Fulvifomes* clade which, besides species of *Fulvifomes*, also included *Inonotus luteoumbrinus* (Romell) Ryvarden and *I. porrectus* Murrill. Our studied samples, JV1408/4, JV0611/III, and JV0611/8P (as *Fulvifomes centroamericanus*) formed a strongly supported lineage; and JV0904/1, JV0312/24.10-J and JV1008/21 (*Fulvifomes krugiodendri*) formed another supported lineage.

In the phylogeny inferred from the ITS sequences (Fig. 2), four newly sequenced specimens (JV0904/1, JV0312/24.10-J, JV1008/21 as *Fulvifomes krugiodendri* and JV0611/III as *Fulvifomes centroamericanus*) clustered together with all sampled species of *Fulvifomes* and *Inonotus porrectus* in the *Fulvifomes* clade. The two new species formed lineages that had full statistical supports and were separated from other sampled species.

Taxonomy

***Fulvifomes centroamericanus* Y.C. Dai, X.H. Ji & Vlasák, sp. nov.**
MycoBank: MB818638
Figs 3, 4

Holotype. GUATEMALA. Tikal, 4 Nov 2006, leg. J. Kout, on living angiosperm tree, JV0611/III (Holotype in JV, isotype in BJFC).

Etymology. *Centroamericanus* (Lat.): referring to the distribution of species.

Description. Basidiocarps perennial, sessile, broadly attached, solitary, without odor or taste, woody hard, light in weight when dry. Pilei dimidiate, applanate, projecting up to 15 cm, 20 cm wide and 8 cm thick at the base. Pileal surface dark grey, crusted, uncracked; margin cinnamon-buff, obtuse. Pore surface pale yellow, shining; sterile margin distinct, yellowish brown, up to 3 mm wide; pores circular, 8–10 per mm; dissepiments thick, entire. Context yellowish brown, woody hard, up to 5 cm thick. Tubes yellowish brown, woody hard, up to 3 cm thick, tube layers distinctly stratified with intermittent context layers, individual tube layer up to 2 mm thick.

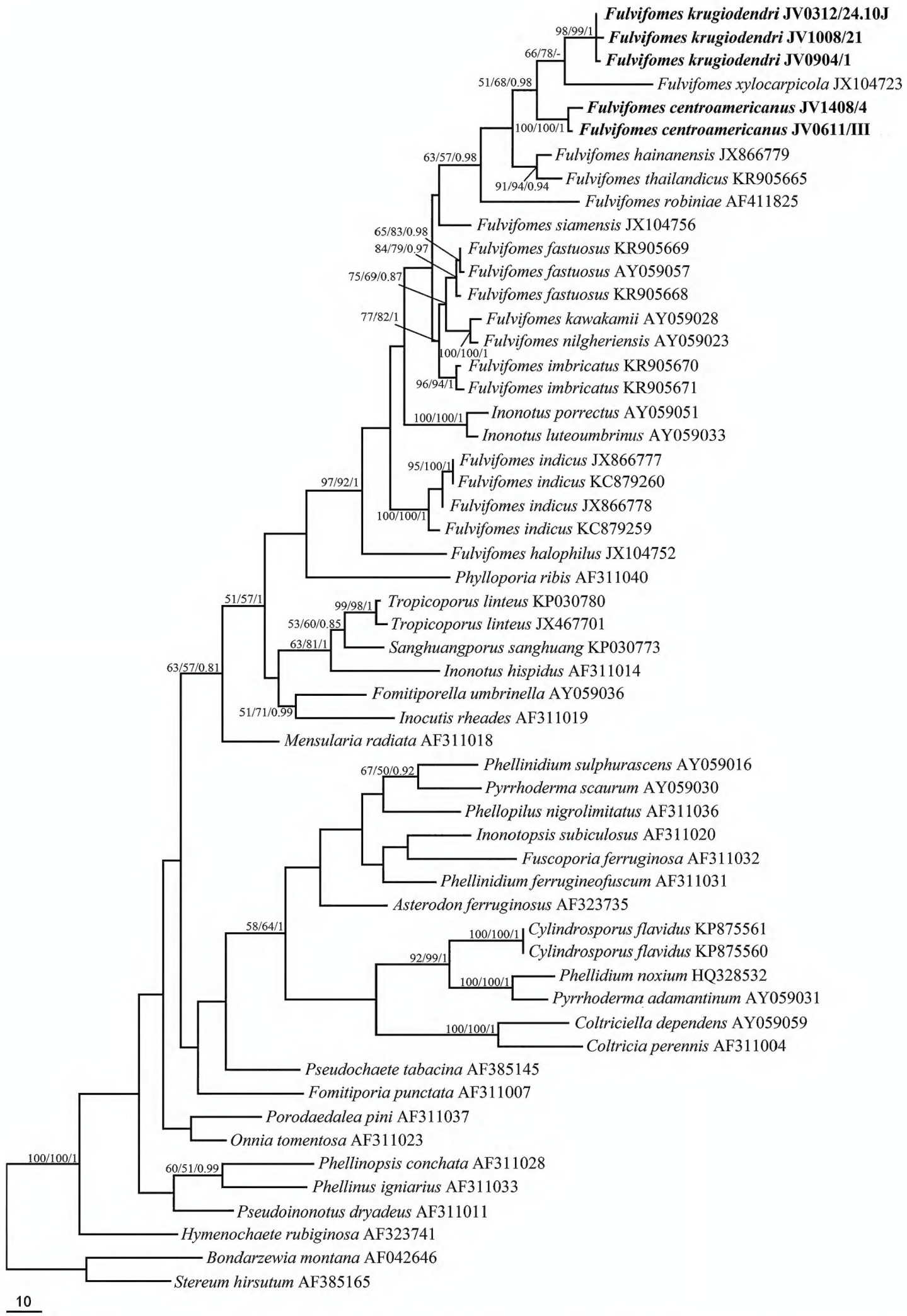

Figure 1. Phylogeny of *Fulvifomes* inferred from nLSU dataset. Topology is from MP tree and statistical values (MP/ML/BI) are indicated for each node that simultaneously received BS from ML and MP not below 50%, and BPP from BI not below 0.8.

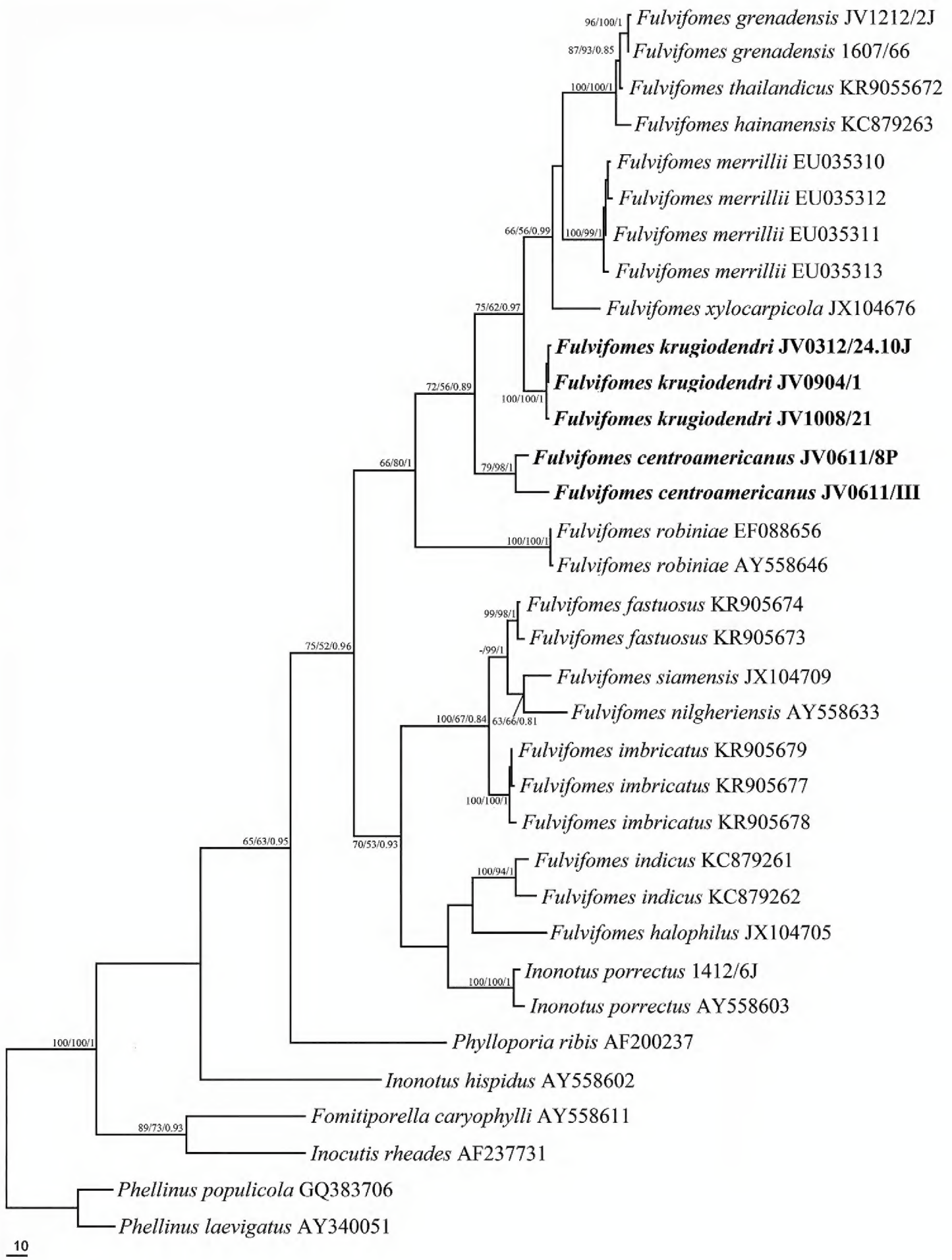

Figure 2. Phylogeny of *Fulvifomes* inferred from ITS dataset. Topology is from MP tree and statistical values (ML/MP/BI) are indicated for each node that simultaneously received BS from ML and MP not below 50%, and BPP from BI not below 0.8.

Figure 3. A basidiocarp of *Fulvifomes centroamericanus*. Scales bar: 10 mm.

Hyphal structure. Hyphal system dimitic; generative hyphae simple septate; skeletal hyphae dominant; tissue darkening but otherwise unchanged in KOH.

Context. Generative hyphae yellowish, slightly thick-walled, unbranched, frequently simple septate, 2–3.5 µm in diam; skeletal hyphae dominant, pale yellow to brown, thick-walled with a wide lumen, unbranched, aseptate, regularly arranged, 3–5 µm in diam.

Tubes. Generative hyphae hyaline to pale yellow, thin- to slightly thick-walled, rarely branched, frequently simple septate, 1.5–3 mm in diam; skeletal hyphae dominant, pale yellow, thick-walled with a wide to narrow lumen, rarely branched, aseptate, interwoven, 2–3 mm in diam. Setae absent; cystidia and cystidioles absent; hymenium collapsed, basidia and basidioles not obserbed.

Spores. Basidiospores subglobose, yellowish brown, thick-walled, smooth, usually collapsed when mature, IKI–, CB–, (3.8–)3.9–4.5(–4.6) × (3.5–)3.7–4.2 µm, L = 4.11 µm, W = 3.92 µm, Q = 1.04–1.05 (n = 60/2).

Additional specimen examined (paratypes). COSTA RICA. Las Pailas Ranger Station, Rincon de la Vieja, July 2016, on living angiosperm tree, JV1607/90 (JV); 1 Aug 2014, on living angiosperm tree, JV1408/4 (JV, BJFC). GUATEMALA. Uaxactún, 2 Nov 2006, leg. J. Kout, on living angiosperm tree, JV0611/8P (JV).

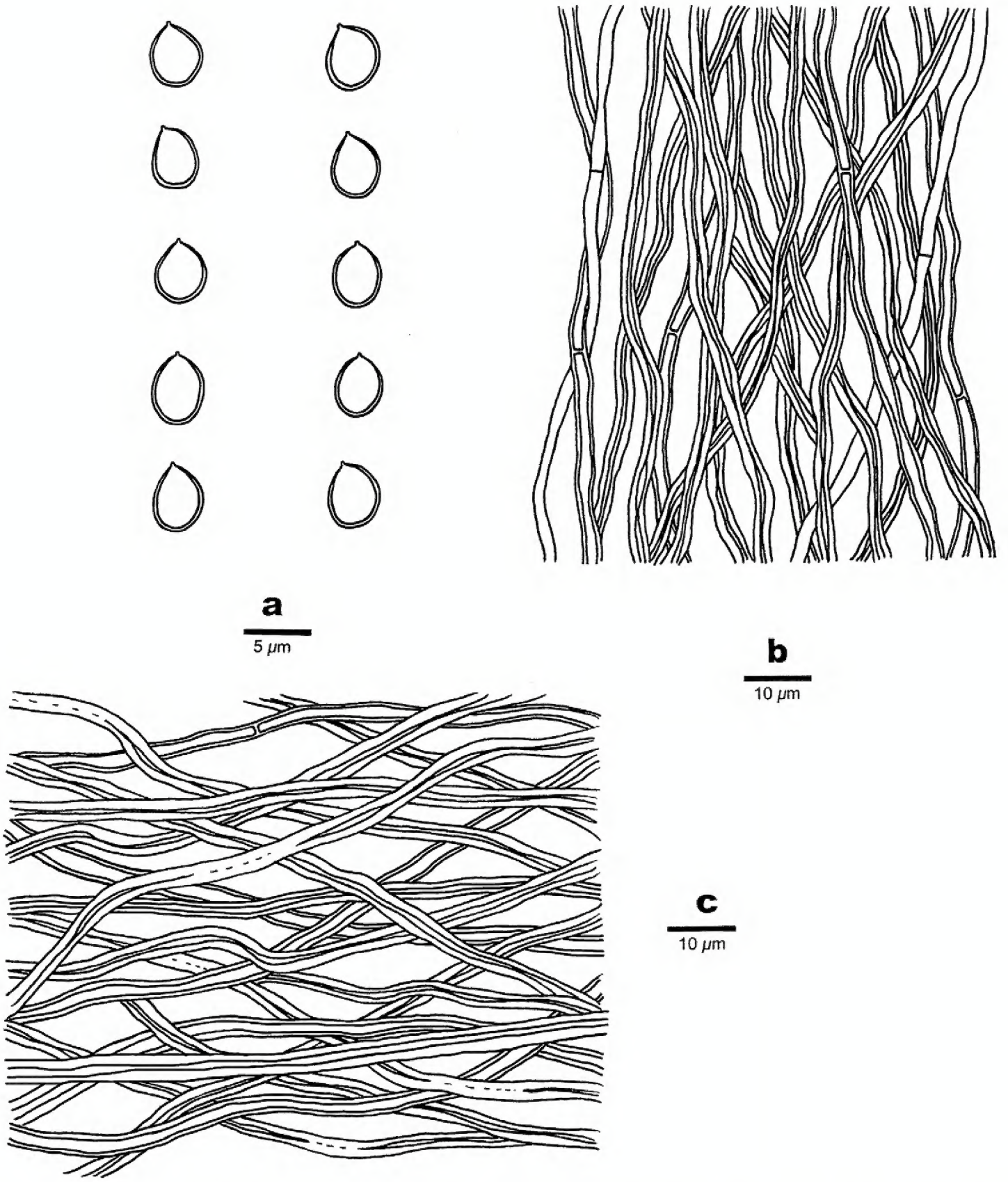

Figure 4. Microscopic structures of *Fulvifomes centroamericanus*. **a** Basidiospores **b** Hyphae from trama **c** Hyphae from context.

Fulvifomes krugiodendri **Y.C. Dai, X.H. Ji & Vlasák, sp. nov.**
MycoBank: MB818639
Figs 5, 6

Holotype. USA. Florida: Miami, Matheson Hammock, 19 Apr 2009, on living tree of *Krugiodendron ferreum*, *JV0904/1* (Holotype in PRM, isotype in JV and BJFC).

Figure 5. A basidiocarp of *Fulvifomes krugiodendri*. Scales bar: 10 mm.

Etymology. *Krugiodendri* (Lat.): referring to the host tree genus *Krugiodendron*.
Description. Basidiocarps perennial, sessile, solitary, without odor or taste, woody hard, light in weight when dry, projecting up to 10 cm, 15 cm wide and 6 cm thick at center. Pileal surface dark grey, crusted, concentrically sulcate with narrow zones, cracked; margin cinnamon-buff, obtuse. Pore surface grayish brown, shining; sterile margin distinct, yellowish brown, up to 3 mm wide; pores circular, 7–9 per mm; dissepiments thick, entire. Context dark brown, woody hard, up to 5 mm thick. Tubes yellowish brown, woody hard, up to 5.5 cm thick, tube layers distinctly stratified with intermittent context layers, individual tube layer up to 3 mm thick.

Hyphal structure. Hyphal system dimitic; generative hyphae simple septate; skeletal hyphae dominant; tissue darkening but otherwise unchanged in KOH.

Context. Generative hyphae pale yellowish, slightly thick-walled, rarely branched, occasionally simple septate, 2–3 μm in diam; skeletal hyphae dominant, yellow to brown, thick-walled with a wide to narrow lumen, unbranched, aseptate, interwoven, 3–4 μm in diam.

Tubes. Generative hyphae hyaline to pale yellow, thin- to slightly thick-walled, rarely branched, frequently simple septate, 1.5–3 μm in diam; skeletal hyphae dominant, pale yellow, thick-walled with a wide to narrow lumen, unbranched, aseptate, loosely interwoven, 2–3 μm in diam. Setae absent; cystidia and cystidioles absent; hymenium collapsed, basidia and basidioles not oberved.

Spores. Basidiospores subglobose, yellowish brown, thick-walled, smooth, some collapsed when mature, IKI–, CB–, (4.0–)4.3–5.0(–5.1) × (3.7–)4.0–4.5(–4.8) μm, L = 4.60 μm, W = 4.21 μm, Q = 1.08–1.09 (n = 60/2).

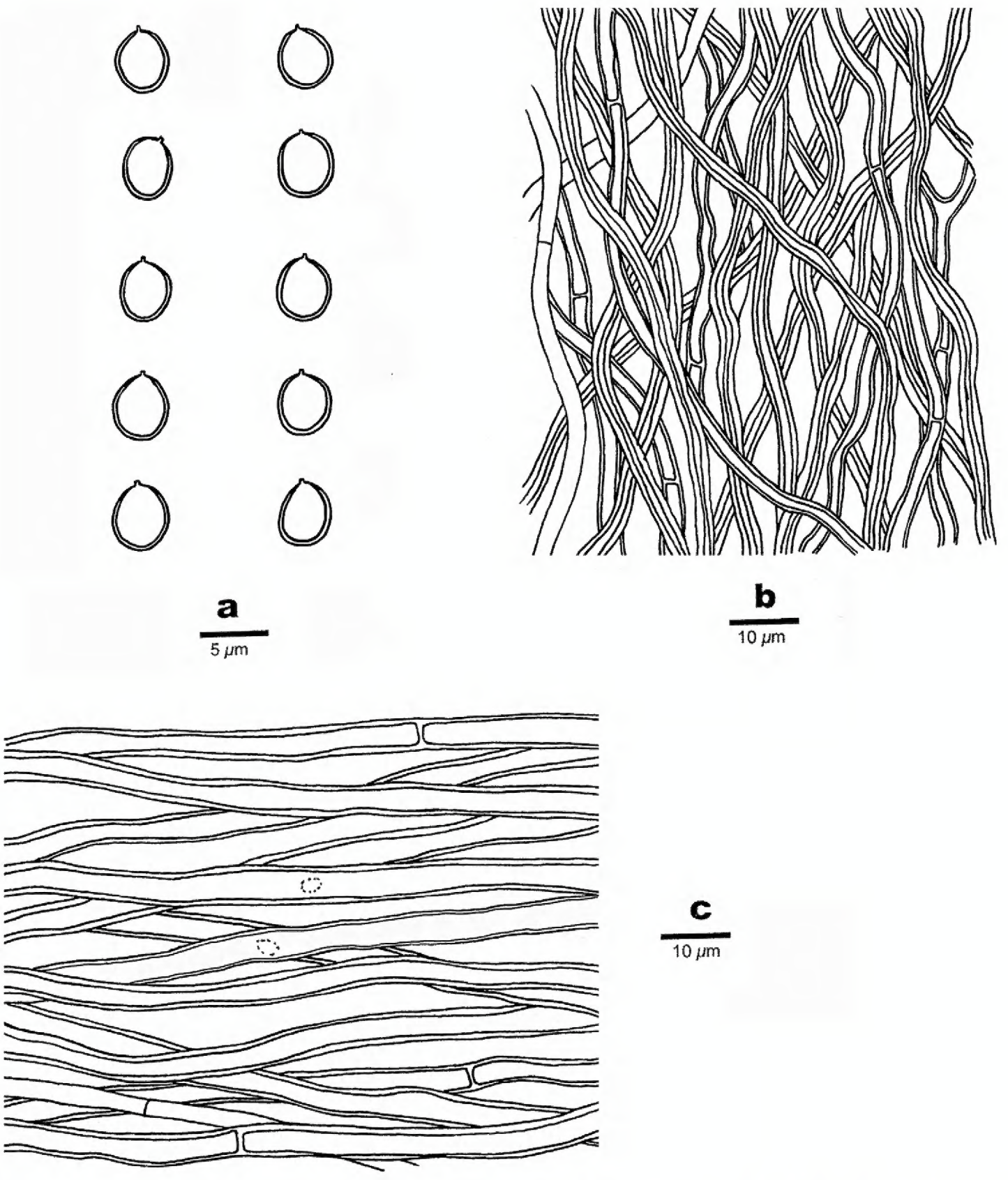

Figure 6. Microscopic structures of *Fulvifomes krugiodendri*. **a**: Basidiospores **b** Hyphae from trama **c** Hyphae from context.

Additional specimens examined (paratypes). USA. Florida: Miami, Matheson Hammock, 24 Dec 2003, on living tree of *Krugiodendron ferreum*, JV0312/24.10-J (JV, BJFC), August 2010, JV1008/21 (JV, BJFC).

Discussion

Fulvifomes krugiodendri and *F. centroamericanus* fit well in *Fulvifomes* (emended by Zhou 2014) with perennial and pileate basidiocarps with a homogeneous, dimitic hyphal system, lack of hyphoid and hymenial setae, and subglobose, yellowish to brown and thick-walled basidiospores. Besides, they formed distinct lineages within the *Fulvifomes* clade in the phylogenies inferred from nLSU and ITS datasets (Figs 1 and 2).

Fulvifomes centroamericanus is mostly similar to *F. robiniae* by sharing applanate basidiocarps and subglobose basidiospores, which are sometimes collapsed on one side (Gilbertson and Ryvarden 1987), and phylogenetically both species are closely related. However, *F. centroamericanus* is distinct by smaller basidiospores (3.9–4.5 × 3.7–4.2 µm), whereas *F. robiniae* has larger basidiospores (5–6 × 4.5–5 µm; Gilbertson and Ryvarden 1987). Moreover, according to Zhou (2015), *F. robiniae* has abundant branched skeletal hyphae in trama and a monomitic hyphal system in the context, which differ from *F. centroamericanus* having unbranched skeletal hyphae in trama and a strictly dimitic system.

Fulvifomes krugiodendri resembles *F. merrillii* (Murrill) Baltazar & Gibertoni by producing perennial and ungulate basidiocarps, concentrically sulcate pileal surface when mature, and an obtuse margin (Ryvarden 2004). However, *F. merrillii* microscopically produces bigger basidiospores (5–6 × 4–5 µm, Ryvarden 2004), while they are 4.3–5.0 × 4.0–4.5 µm in *F. krugiodendri*. Moreover, *F. merrillii* has reddish brown to dull brown and matted to rugose upper surface (Ryvarden 2004) and *F. krugiodendri* has black and crusted upper surface.

Phylogenetically, *Fulvifomes krugiodendri* is closely related to *F. xylocarpicola, F. thailandicus* and *F. hainanensis* (Figs 1 and 2), the latter three species were recently described from Thailand and China (Hattori et al. 2014, Zhou 2015). *F. krugiodendri* and *F. xylocarpicola* resemble each other by similar hyphal structures and basidiospores (4–5.5 × 3.5–4.5 µm in *F. xylocarpicola*; Hattori et al. 2014), but *F. xylocarpicola* differs from *F. krugiodendri* in its uncrust basidiocarps and the bigger pores (4–6 per mm). The morphological characters of *F. krugiodendri* and *F. thailandicus* are different. *F. thailandicus* has broadly attached, dimidiate, applanate basidiocarps, bigger pores (6–7 per mm) and larger basidiospores (4–5.8 × 4.1–4.8 µm, Zhou 2015). *F. krugiodendri* resembles *F. hainanensis* by producing perennial and ungulate basidiocarps, but the latter has duplex context and uncracked pileal surface.

Inonotus porrectus Murrill and *I. luteoumbrinus* (Romell) Ryvarden are nested within the *Fulvifomes* clade (Figs 1 and 2). Data from Sakayaroj et al. (2012) indicated that *I. luteoumbrinus* and *I. porrectus* fell into the *Fulvifomes* clade. However, both species have annual and soft fruiting bodies, a monomitic hyphal structure, and dark brown to black basidiospores, these characters do not correspond to *Fulvifomes,* and for the time being we still keep them in *Inonotus* P. Karst.

Lack of setae and relatively uniform basidiospores (shape, color and size) in most *Fulvifomes* species reduce the number of scorable traits substantially so that the mor-

phological determination is problematic. *Fulvifomes fastuosus* (type from Singapore) and *F. merrillii* (type from Philippines) were described from tropical Asia and their similar kins from Central and South America were simply classified under these old names because of lack of discriminating characters. Nevertheless, our broadly based sequencing of about 50 recent collections from Central America (not shown in the Figs 1 and 2) revealed *F. fastuosus* or *F. merrillii* not existed in Central America but several more or less distinct clades of related but different species which will be published in the coming papers.

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